

Seasonality alters drivers of soil enzyme activity in subalpine grassland soil undergoing climate change



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ABSTRACT

In mountain ecosystems with marked seasonality, climate change can affect various processes in soils, potentially modifying long-term key soil services via change in soil organic carbon (C) storage. Based on a four-year soil transplantation experiment in Swiss subalpine grasslands, we investigated how imposed climate warming and reduced precipitation modified the drivers of soil carbon enzyme potential activities across winter and summer seasons. Specifically, we used structural equation models (SEMs) to identify biotic (microbial community structure, abundance and activity) and abiotic (quantity and quality of organic matter resources) drivers of soil C-enzymes (hydrolase and oxidase) in two seasons under two different climate scenarios. We found contrasting impacts of the climate manipulation on the drivers of C-enzymes between winter and summer. In winter, no direct effect of climate manipulation (reduced rainfall and warming) on enzyme activity was observed. Yet, climate indirectly down-regulated enzyme activity through a decrease in the availability of water extractable organic carbon (WEOC) labile resources. During summer, reduced soil moisture –induced by the climate manipulation– directly reduced soil microbial biomass, which led to a decrease in C-enzyme activity. In general, across both seasons, neither microbial community structure, nor organic matter quality were strong determinants of enzymatic activity. In particular organic matter recalcitrance (aromaticity) was not found as a general driver of either hydrolase or oxidase C-enzyme potential activities, though we did observe higher C-enzyme activities led to an increase of particulate organic matter recalcitrance in the summer season. Overall, our results highlight the seasonality of climate change effects on soil organic matter enzymatic decomposition, providing a comprehensive picture of seasonal potential cause and effect relationships governing C mineralization in subalpine grasslands.

1. Introduction

Soils store vast amounts of carbon (C) as soil organic matter (SOM), which equals, if not exceeds, the collective C stock in the atmosphere and vegetation (IPCC, 2013). Soil microbial communities play a key role in SOM decomposition processes, annually releasing ca. 60 GtC as respired CO₂ into the atmosphere (IPCC, 2013; Lal, 2008), or roughly double the anthropogenic greenhouse gas contribution. To decompose

SOM, soil microorganisms release soil extracellular enzymes, which break down SOM through hydrolytic or oxidative processes (Burns et al., 2013; Sinsabaugh, 2010). This enzymatic depolymerisation process is a crucial step as it has been hypothesized to be the rate-limiting step in SOM decomposition processes, thus controlling C storage in soil (Bengtson and Bengtsson, 2007; Conant et al., 2011). In a warmer world, kinetic theory predicts enzyme activities to increase (Davidson and Janssen, 2006). In soil, however, enzyme activity rates are thought

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to be primarily determined by the frequency of substrate-enzyme interactions (Conant et al., 2011). The probability for enzymes to interact with substrates is controlled by a combination of biological, physical and chemical drivers (Dungait et al., 2012) which correspond mainly to (i) the quantity and turnover of the enzyme pool produced by microbial communities, (ii) the chemistry and availability/protection of OM substrates and (iii) the soil moisture and temperature conditions that define the physical conditions in which enzymes operate. However, it is difficult to understand the effects of climate change on all of these factors combined. Explicit consideration of both direct and indirect impacts of climate change on soil microorganisms and organic matter protection are required to understand complex interactions and feedbacks (Bardgett et al., 2008; Schmidt et al., 2011).

Mountain ecosystems cover 12.3% of all terrestrial land area and store large amounts of soil organic carbon as decomposition processes are limited by cold temperatures (Körner et al., 2011; Houghton, 2007; Wohlfahrt et al., 2008). These regions are currently experiencing strong climatic changes with alterations in temperatures, precipitation and seasonal intensity and duration (Gobiet et al., 2014). Moreover mountain areas offer an opportunity to test the impact of climate change as elevation gradients represent natural climate change experiments ideally suited to predicting future climate scenarios (Körner, 2007).

Future climate change scenarios for the European Alps predict an increase in mean annual temperature (MAT), together with a decrease in snow cover in winter and an increase in the frequency of extreme events such as drought and heat waves in summer (C2SM, 2011; IPCC, 2013). Such changes have already been reported to strongly alter the drivers of soil potential enzyme activities (Henry, 2013). Climate change, particularly warming and drought, is expected to affect the dynamics of soil microbial communities, organic substrate availability and therefore enzyme decomposition kinetics (Allison and Vitousek, 2005; Conant et al., 2011; Davidson and Janssens, 2006). Although we largely understand the impact of climate on microbial communities and OM substrate availability, a key knowledge gap remains to understand how changing ecological conditions affect interactions between microbial communities and substrate availability in driving C-degrading enzyme activities. This needs addressing urgently in order to build a framework to predict the future capacity of soils to act as a C sink (Sinsabaugh, 2010).

This study therefore aims to determine the effect of climate change on multiple interactive drivers of C-enzyme activities in winter and summer seasons in a subalpine grassland. We sought to perform an integrative analyses on previously published datasets from an altitudinal transplant experiment (moving soil turves to a lower altitude) with detailed data on soil microbial activity, abundance and structure; as well as SOM organic matter resources availability and chemistry (Puissant et al., 2015, 2017) collected after four years of imposed climate change. Structural equation modelling (SEM) based on path analysis have been used to evaluate how climate change influenced the interactions between microbes and SOM protection that driven C-enzyme potential activities. The climate change manipulation led to a discontinuous and thinner snow cover in winter and a warmer and drier climate in summer seasons. The effect of the climate change manipulation on the drivers of C-enzymes potential activities were evaluated separately in winter and the summer seasons to specifically examine different seasonal drivers. Our specific objectives were to (i) evaluate how the climate change manipulation affected C-degrading enzyme potential activities (hydrolase and oxidase) due to direct effects on microbial communities as well as effects on SOM resource availability and chemistry; and (ii) to determine whether the effects were consistent across seasons (winter vs summer).

2. Materials and methods

2.1. Study site and experimental manipulations

The experiment was located in the Swiss Jura mountain range and consisted of a high-to-low elevation soil translocation. Our highest site (1350m a.s.l., Combe des Amburnex, N 46°54', E 6°23') acted as the donor site. Its long-term mean annual temperature is +4.5 °C and mean annual rainfall is 1750 mm, which includes over 450 mm of snow. Combe des Amburnex is a species rich grassland and the soil type is Cambisol (IUSS Working Group WRB, 2007) on Jurassic limestone with an organic carbon content of 77 g kg⁻¹ in average (Puissant, 2015).

We performed a four-year climate manipulation experiment which simulated a year-round intensive climate change scenario, expected regionally within the 21st century (A2 scenario, Meehl et al. 2007) aiming an average of 4 °C (MAT, +4 °C) temperature increase and 40% decrease in precipitation (MAP, -40%) (Gavazov et al., 2013). From the donor site (Combe des Amburnex), ten monoliths of undisturbed soil (30 cm depth) and its vegetation were placed in rectangular PVC boxes (60 × 80 and 35 cm in height), further referred to as mesocosms. Five mesocosms were placed back in their home site, i.e. at the same altitude (control, 1350 m a.s.l.), whilst the remaining five mesocosms were brought to a lower-altitudinal site (570 m a.s.l., Arboretum d'Aubonne, N46°51', E6°37') to simulate the envisaged climate scenario. All mesocosms were placed in pre-dug pits.

In the winter and summer season of the fourth year of the transplantation experiment, five intact soil cores (5 cm diameter × 10 cm length), i.e. one core per replicate mesocosm, were taken, placed in a cool box, and transported to the lab before analysis.

2.2. Soil microclimate

Soil temperature within the topsoil horizon were recorded every minute in each mesocosm, using Em50 data-loggers (Decagon Devices, Inc., USA) coupled to ECH2O EC-TM probes inserted at 3 cm depth. The gravimetric soil water content was measured by drying soil at 105 °C for 48 h according to norm NF ISO 16586 (2003). Winter sampling (February 20th, 2013) corresponded to the maximum snow cover at the control high elevation site, whereas at the low elevation site (570 m a.s.l.), the snow cover had melted completely several times during the winter, resulting in strong mid-winter soil temperature fluctuations. The daily average soil temperature at 3 cm depth within the mesocosms was 0.6 and 1.2 °C and the gravimetric soil moisture content 50% and 43% at the high and low elevation sites, respectively (Puissant et al., 2015). Summer sampling (September 2nd, 2013) corresponded to a dry period at the end of summer with an average soil temperature at 3 cm depth of 13.2 and 18.4 °C and gravimetric soil moisture of 33% and 21% at the high and low elevation sites, respectively. Overall, our climate manipulation increased the mean annual soil temperature by 4 °C (November 2012 to October 2013).

2.3. Soil analysis

For all chemical soil analyses, samples were dried at 40 °C as indicated in norm NF ISO 11464 (2006). In order to identify the effect of climate change on the drivers of potential C-enzymes activities with a structural equation modelling (SEM) approach, we used published data on the effect of the climate manipulation on (i) soil microbial activity, abundance and structure (Puissant et al., 2015) and on (ii) SOM organic matter resources availability and chemistry (Puissant et al., 2017). Data used to perform SEMs are summarized in Table 1. Details on each method performed to obtain all the variables used for SEM models can be found in Supplementary material.

Table 1
Variables used for performing Structural Equation Models (SEMs). These data are derived from two previous studies on the same experiment focus on either, (i) microbial abundance, structure and activity (Puissant et al., 2015) or, (ii) soil organic carbon pools contribution and chemistry (Puissant et al., 2017). 1 → MUB: 4-methylumbelliferone; 2 → ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.

Measurements	Type of SEM	Variable name used in SEMs	Units	Annual average and standard error	Ecological function	Description	Sources methods
Climate conditions							
Soil moisture	Abundance & Compositional	Soil moisture	%	31 ± 16	Climate change manipulation proxy	Gravimetric soil water content	NF ISO 16586 (2003)
Soil enzymes activities							
Cellobiohydrolase; β-glucosidases; xylosidase; lipase	Abundance & Compositional	C-Hydrolase	nmol of product per second per g of dry soil	4.86 ± 0.9	C-substrate enzymes activity	Fluorogenic methods using 4-MUB	According to Marx et al. (2001) with small modifications Floch et al., 2007
Phenol oxidase		C-Oxidase		0.65 ± 0.57		Oxidation of ABTS for phenol oxidase	
Microbial population characteristics							
Microbial Biomass	Abundance	MB	mg C/gsoil	3.98 ± 1.8	Abundance of decomposer community	Chloroform fumigation extraction	Brookes et al., 1985; Vance et al., 1987
PLFA	Compositional	MCS1 and MCS2		−0.8 ± 1.1 and 0.4 ± 5	Proxy for the structure of decomposer community	Two first axis of a PCA on microbial phospholipid fatty acid data (Puissant et al., 2015)	According to Bligh and Dyer (1959) and modified by Borjesson et al. (1998)
SOM resources quantity (physical fractions)							
Water Extractable Organic Carbon (WEOC)	Abundance	WEOC	mg C/g of dry soil	0.12 ± 0.04	Substrate already available for decomposer	Water extraction filtered at 0.45 μm	Zsolnay et al. (2003) with small modifications
free Particulate Organic Matter (freePOM)		freePOM	g C/kg of dry soil	6.8 ± 5.4	Labile pool of OM	Density fractionation (1.6 g.cm ⁻³)	Leifeld et al. (2005, 2009)
Occluded Particulate Organic Matter (occPOM)		occPOM		6.95 ± 2.1	Labile pool of OM but protected by soil macro-aggregates	Density fractionation and macro-aggregates disruption with ultrasonication (22 J.mL ⁻¹)	and Zimmermann et al. (2009)
SOM resources quality							
WEOC chemistry	Compositional	UV280	Relative absorbance	0.08 ± 0.04	WEOC Aromaticity estimating its biodegradability	Ultraviolet (UV) spectroscopy at 280 nm	Kalbitz et al., 2003
POM Aromaticity index		POM aromaticity	Absorbance	6.3 × 10 ⁻³ ± 1.4 × 10 ⁻³	POM Chemistry estimating its biodegradability	Mid-infrared (MIR) spectroscopy spectral region corresponding to aromatic C=C bonds 1,576–1,618 cm ⁻¹	Pengerud et al. (2013) and Robroek et al. (2015)

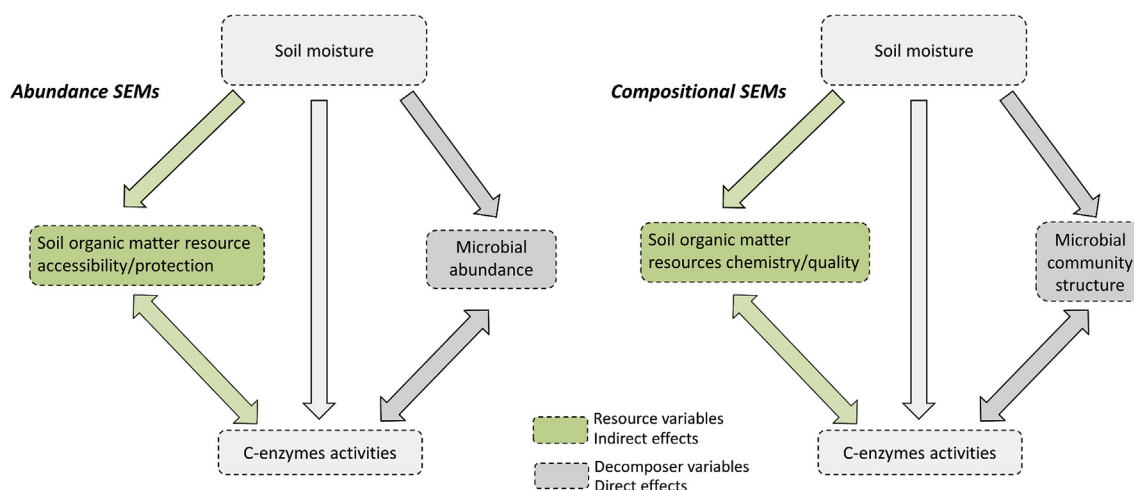


Fig. 1. Scheme of the conceptual and hypothetical path-relation network used to perform SEMs. Green arrows indicate paths involving change in soil organic matter resource quality or quantity. Grey arrows indicate paths involving change of soil microbial community abundance or structure. Double headed arrow indicate that the causal path has been tested in the two direction in two separated different SEM. Abundance SEM and compositional SEM models are the two main kind of SEM performed based on quantity data or quality data. Details of the variables used are given in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Structural equation modelling (SEM)

We organized the dataset into a path-relation network subjected to structural equation modeling (Fig. 1) so as to identify the main seasonal drivers of SOM enzymatic decomposition in subalpine grasslands that were modified by climate change (see e.g. Grace et al., 2014).

Following current concepts of the SOM enzymatic decomposition processes, we proposed an *a priori* SEM model of hypothesized relationships within a path diagram allowing a causal interpretation of SEM outputs (Grace et al., 2012).

We chose soil moisture as an exogenous continuous variable in the SEM analyses in order to reflect within and between treatment natural variability. Soil moisture can be considered an integrated proxy to climate change as it reflects ambient air temperature, precipitation and evapotranspiration (Seneviratne et al., 2006). Indeed, soil gravimetric moisture and soil temperature were strongly correlated (Pearson $R^2 = 0.94$ and p -value < 0.001) within the mesocosm curves. The variance in soil gravimetric moisture was largely explained by our climate change manipulation ($R^2 = 0.53^*$ and $R^2 = 0.59^{**}$; linear model for winter and summer season respectively) confirming that this variable integrates the effect of the climate change manipulation. Moreover, previous investigations of the same soil transplantation experiment revealed the prevailing soil moisture vs temperature controls on soil C turnover (Mills et al., 2014) and (Gavazov et al., 2014). C-enzymes potential activities were split into hydrolase enzymes (mean of β -glucosidases, cellobiohydrolase, xylosidase, lipase) and oxidase enzyme (phenol oxidase) (Table 1). Oxidases are less stable in the environment than extracellular hydrolase enzymes and could also respond differently to climate change (Singsabaugh, 2010). Potential drivers of C-enzymes activity were divided into “decomposer variables” (abundance and composition of microbial communities) and “resource variables” including (i) the abundance of water extractable organic carbon fraction (WEOC) and of free and intra-aggregate particulate organic matter (freePOM and occPOM), and (ii) the chemical composition of SOM fractions estimated by several spectroscopic indices (infrared spectroscopic indices for POM fractions and an ultraviolet spectroscopic index for the WEOC fraction, see Fig. 1 and Table 1).

2.5. SEM building

To understand whether the effects of our climate change manipulation on the drivers of SOM enzyme decomposition diverged between

winter and summer, SEMs were performed separately for the two seasons. For each season, two individual SEM path analysis models were built: (i) an ‘abundance SEM’ model based on the abundance of microbial decomposers and SOM resources; (ii) a ‘compositional SEM’ model based on the PLFA-derived structure of microbial decomposers community and the chemistry of SOM resources (Fig. 1). PLFA data were summarized using the two axis of the principal component analysis (Puissant et al., 2015; Supplementary material). From the conceptual metamodel and initial SEMs (Figs. 1 and 2, Fig. 3) we identified the key pathways and C-enzyme drivers by model simplification using step-wise exclusion of variables with non-significant regression weights and covariances (Milcu et al., 2013). Significant SEMs but with weaker model fit are presented in supplementary material. All SEM analyses were conducted using the *sem* R package (Fox, 2006). Adequate model fit was identified by non-significant chi-square tests ($P \geq 0.05$), low Akaike Information Criterion (AIC), low Root Mean Square Error of Approximation index ($RMSEA \leq 0.1$), low Standardized Root Mean Square Residual index ($SRMR \leq 0.1$), and high Comparative Fit Index ($CFI \geq 0.90$) (Grace et al., 2014). Due to non-satisfying fit indices, no compositional SEM was retained for the winter season.

3. Results

3.1. Climate change impact on C-enzymatic drivers in winter season

In winter, abundance SEM path analysis showed that decreased soil moisture content led to a reduction in the amount of water extractable available carbon (WEOC). The activity of both hydrolase and oxidase enzymes were significantly affected by the amount of WEOC available (Fig. 4A). The amount of POM fractions was not a significant driver of C-related enzyme potential activities. Interestingly soil moisture did not predict directly the amount of microbial biomass, but higher C-hydrolase activity led to an increase in microbial biomass.

Overall, in winter, the abundance SEM (Fig. 4A) showed that lower moisture content was associated with lower enzyme potential activities and microbial biomass when the amount of directly available carbon decreased (WEOC). In winter the climate change manipulation led to a decrease of soil moisture at the lower elevationsite with -21% moisture content decreased compare to the control site (Table 2).

The SEM based on compositional data (Fig. 4C) failed to converge, which means that a stable solution has not been reached. Neither the chemistry of SOM resources (WEOC and POM fractions), nor the

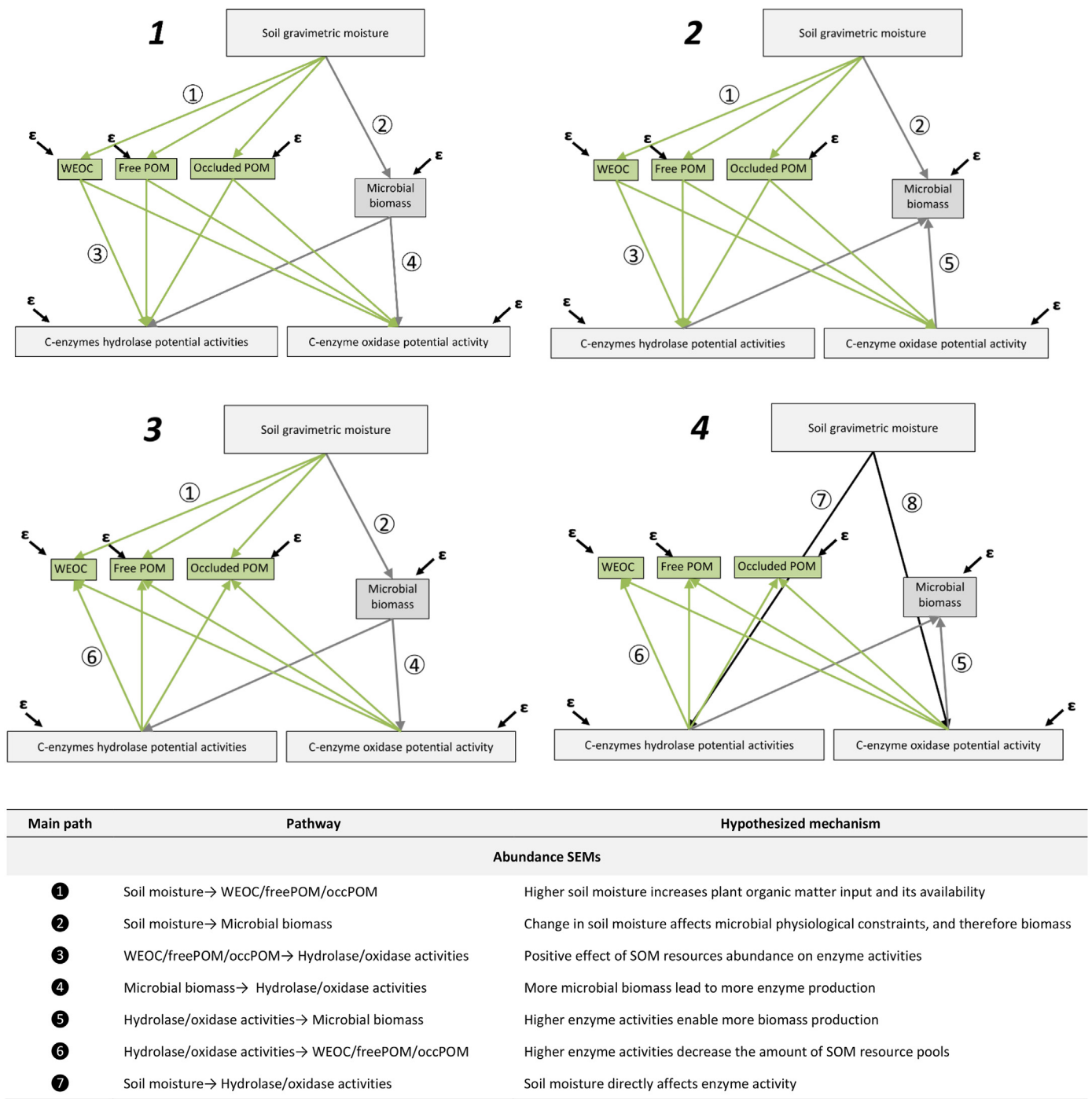


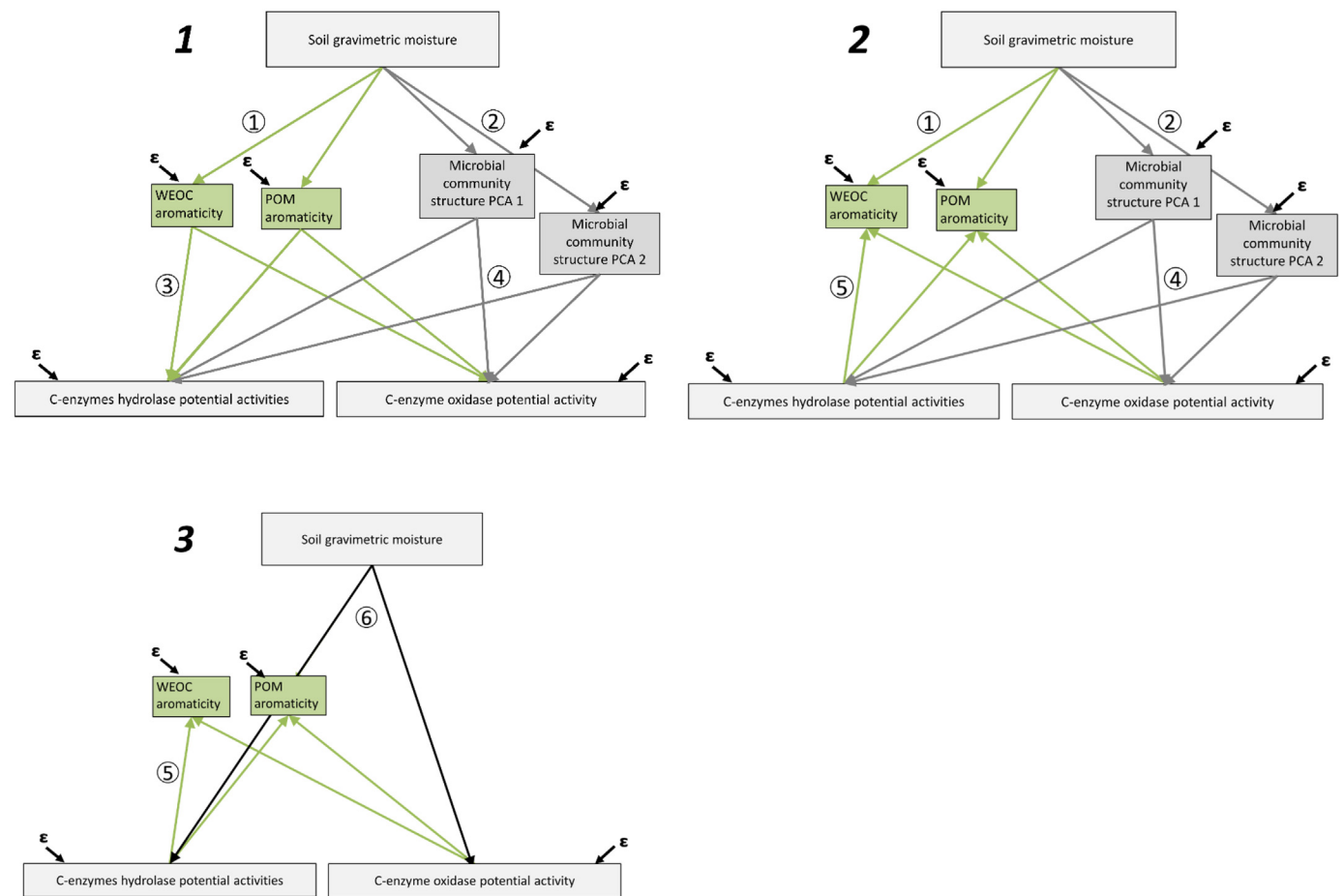
Fig. 2. Abundance initial SEMs showing the different path-relation network used to perform SEMs. Numbers in circle indicate the hypothesis made behind each causal links. Green arrows indicate paths involving change in soil organic matter resource quantity. Grey arrows indicate paths involving change of soil microbial community abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

structure of microbial community (PLFAs principal component axis) were sufficient to explain the changes in C-enzyme potential activities linked to the climate change manipulation.

3.2. Climate change impact on C-enzymatic drivers in summer season

In summer we observed a direct effect of climate condition (soil moisture) on the microbial community. Indeed, the abundance SEM (Fig. 4B) showed that soil moisture regulated the abundance of soil microbial biomass. Reduced soil moisture content under climate change conditions (-i.e., at lower elevation, -38% moisture content, Fig. 4. B

and Table 2.) led to a decrease in soil microbial biomass. The strong positive relationship between soil moisture and microbial biomass was significantly and explained 0.67 of the variance in microbial biomass (Fig. 4B). Soil microbial biomass was in turn positively controlled by both hydrolase and phenol oxidase enzymes potential activities. Conversely to the winter season we did not observe any effect of SOM resource abundance on C-enzyme activities. Nonetheless, an effect of C-enzyme potential activities was observed on the abundance of the freePOM fraction. Higher C-hydrolase potential activities led to a decrease in the quantity of the freePOM fraction (path coefficient: 0.62**). The summer compositional SEM (Fig. 4D) showed as in the



Main path	Pathway	Hypothesized mechanism
Compositional SEMs		
①	Soil moisture→ WEOC/POM aromaticity	Higher soil moisture changes plant communities, and therefore organic matter input quality
②	Soil moisture→ Microbial community structure	Change in soil moisture affects microbial physiological constraints and therefore microbial community structure
③	WEOC/POM aromaticity→ Hydrolase/oxidase activities	Higher resource aromaticity leads to decreased enzyme activities
④	Microbial community structure→ Hydrolase/oxidase activities	Change in microbial community leads to change in enzyme production
⑤	Hydrolase/oxidase activities→ WEOC/POM aromaticity	Higher enzyme activities leads to increased SOM aromaticity due to preferential degradation of labile resources
⑥	Soil moisture→ Hydrolase/oxidase activities	Soil moisture directly affects enzyme activity

Fig. 3. Compositional initial SEMs showing the different path-relation network used to perform SEMs. Numbers in circle indicate the hypothesis made behind each causal links. Green arrows indicate paths involving change in soil organic matter resource quality. Grey arrows indicate paths involving change of soil microbial community structure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

winter season that SOM resource lability failed to explain C-related enzymes potential activities. However, higher C-hydrolase potential activities were linked to higher soil moisture content (Fig. 4D) and were responsible for an increase of POM aromaticity (path coefficient: 0.67**).

4. Discussion

Climate manipulation (annually reduced precipitation and increased temperature) significantly reduced soil C-enzyme potential activities and the drivers of those changes were found to be strongly

seasonally dependent. Two clearly distinct pathways of C-enzyme drivers were found between the winter and summer seasons. In winter, soil moisture, as affected by climate change manipulation, impacted C-enzyme potential activities indirectly through controlling the resource availability (WEOC). In contrast, in summer soil moisture, as affected by climate change manipulation, directly decreased soil microbial biomass and then led to reduced C-enzyme potential activities. These findings shed light on the importance of considering seasonality to better understand the effect of climate change on C-enzymes potential activities and thus on soil ecosystem processes.

In winter, the climate change manipulation reduced snow cover and

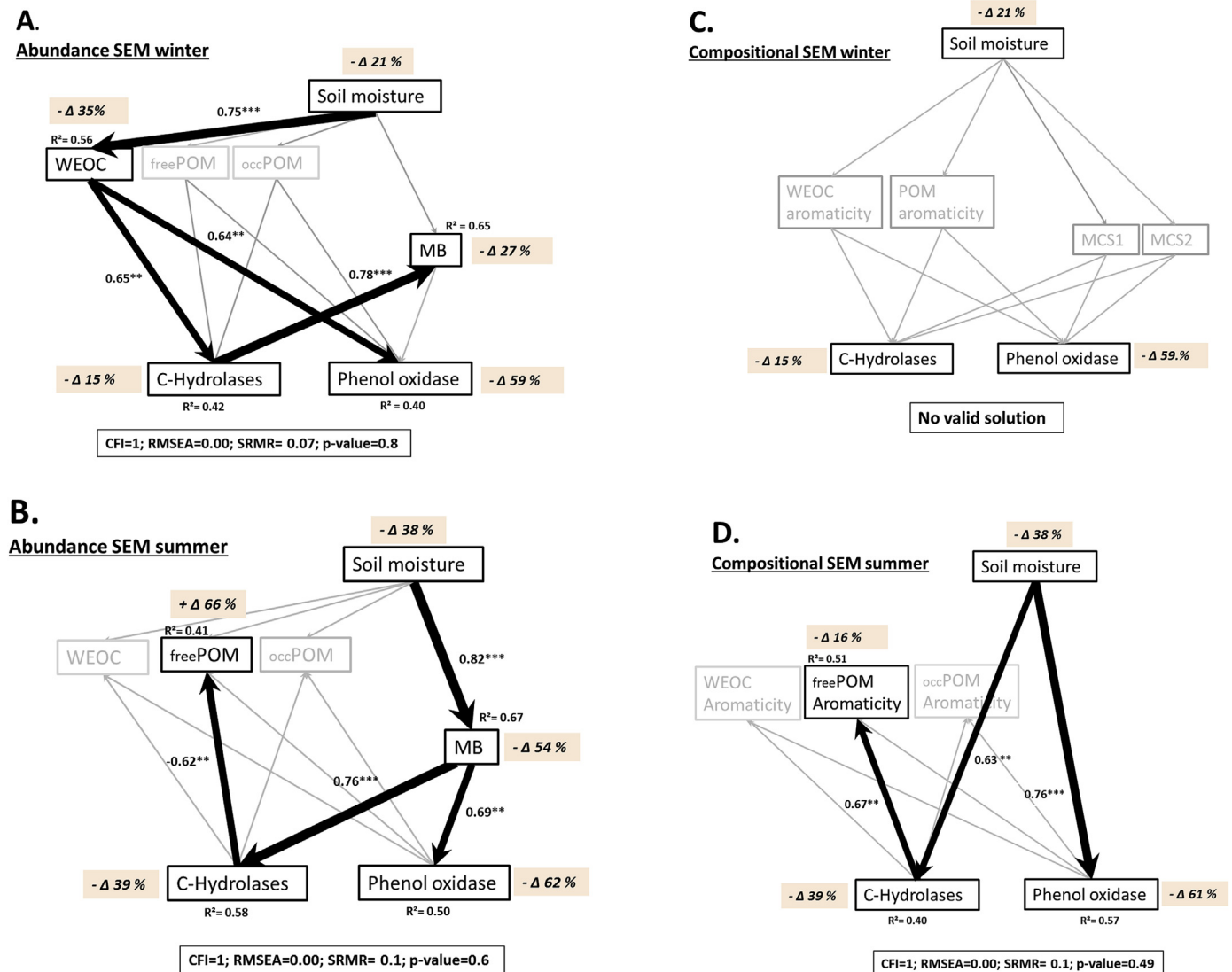


Fig. 4. Seasonal SEMs representing the climate effects on the drivers of SOM enzymatic decomposition. A) Winter abundance SEM, B) Summer abundance SEM, C) Winter compositional SEM, D) Summer compositional SEM. Values in orange boxes indicate delta change between control site (control, 1350 m a.s.l.) and climate manipulation site (570 m a.s.l.). All delta values are expressed as percentage and are positive or negative indicating respectively a relative increase or decrease compared to the control site. Black boxes and arrows indicate significant factors and paths. The boxes and arrows in grey were not significant and were removed from the models. The numbers beside arrows as the arrow width indicates the strength of the effect. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

led to a discontinuous snow cover over the winter period with an overall decrease in soil moisture (Table 2; Puissant et al., 2015). Based on our abundance winter SEM (Fig. 4A), we showed that the consequences of such changes did not directly impact the soil microbial biomass but reduced the amount of organic substrate available, leading to a diminution of C-enzyme potential activities. The reduced hydrolase C-enzyme potential activities under the climate manipulation were strongly linked to the reduction of the microbial biomass. Several studies have reported that soil microbial communities often reached maximal biomass under snow cover (Schadt et al., 2003; Lipson and Schmidt 2004; Gavazov et al., 2017) underlying the crucial role of snow cover in regulating soil microbial abundances. Thermal insulation, soil moisture and organic carbon and nutrient availability have been hypothesized to explain favorable microbial growth conditions under snow cover (Edwards et al., 2007). However, to our knowledge, no studies evaluate the direct and indirect pathways which might explain changes in C-enzyme potential activities and microbial biomass under reduced snow cover. The statistical approach (SEM) chosen in this study

disentangled the direct and indirect effect of climate change manipulation and shed light on the importance role of snow cover for preserving substrate availability (WEOC fraction) for microbial growth. It has been reported that melting of the snowpack coupled with hydrological activity can lead to important losses of nutrient and substrate from the soil system (Edwards et al., 2007). Consistent with our study, Gavazov et al., 2017 found that snow removal decreased SOM mineralization and microbial biomass. In winter, in the subalpine grassland studied, water is not limiting for C-activities and so under these conditions resource availability appeared to limit SOM enzymatic activity (Brooks et al., 2005; Harrysson Drotz et al., 2009; Öquist and Laudon, 2008). Such relationships between microbial activity and abundance and WEOC/DOC content have been reported earlier (Marschner and Kalbitz, 2003; Rees and Parker, 2005), but surprisingly the WEOC degree of aromaticity normally used as a proxy of WEOC biodegradability (Marschner and Kalbitz, 2003) was not found as a driver of soil enzyme activity under the climate change manipulation. The increase in dissolved organic matter leaching observed previously in the same

Table 2

Effect of soil transplantation experiment on the main variable used to build SEMs. The percentage of change from the control site represents for a given variable, the difference between value at the lowest site (570m, Arboretum) corresponding to the climate change scenario simulated versus value at the control site (1350, Marchairuz) expressed as a percent of the control site value. Effect size value is the difference between value at the lowest site (570m, Arboretum) versus value at the control site (1350, Marchairuz) divided by the standard deviation at the control site. Asterisk symbols indicate significant differences (One-way anova) between winter and summer season at each site (· for $p < 0.10$, * for $p < 0.05$, ** for $p < 0.01$; *** for $p < 0.001$).

	Summer				Winter			
	p-value	f-value	% difference from control	effect size	p-value	f-value	% difference from control	effect size
Microbial biomass	**	0.6	−54.4	−1.8	ns	0.0	−27.3	−0.6
WEOC	ns	−0.1	0.3	0.0	·	0.3	−34.6	−1.0
C-enzymes hydrolase	***	0.9	−39.3	−6.7	·	0.2	−15.2	−1.3
WEOC aromaticity	ns	0.1	33.9	0.9	ns	0.0	−20.3	−0.6
C-enzyme oxidase	**	0.6	−62.0	−3.4	**	0.8	−59.4	−2.5
freePOM	*	0.5	66.4	2.7	ns	0.3	64.9	1.8
occPOM	ns	−0.1	−3.3	−0.2	ns	0.0	−11.8	−0.6
Soil moisture	**	0.6	−37.7	−3.0	*	0.5	−21.5	−1.9
Soil temperature	***	0.9	39.2	14.3	ns	0.4	122.3	3.7
PLFA MCS1	ns	−0.1	85.3	−0.3	ns	−0.1	7.6	1.2
PLFA MCS2	ns	0.2	−1533.3	1.2	ns	−0.1	−109.6	0.5
POM aromaticity	ns	0.2	−16.3	−1.7	ns	−0.2	−0.5	0.0

experiment (9.9 mg C L^{−1} under climate change manipulation relative to the control site; Gavazov, 2013) confirms the potential losses of directly available substrate in winter due to climate change and leading to lower C-enzyme potential activities.

Contrastingly, in summer WEOC content was not related to C-enzyme potential activities. Instead, the reduction in soil moisture directly impacted microbial biomass and led to a strong decrease in both hydrolase and oxidase C-enzyme potential activities. The strong gravimetric soil moisture decrease due the climate change manipulation in the summer season (from 34% at the control site to 21% under the climate change condition; delta −38%, Fig. 4B) might have led to a huge water stress for the microbial communities with dehydration and diffusion limiting biological activity (Manzoni et al., 2012). The fact that no organic matter fractions as proxies of resources were found as a driver of C-enzyme potential activities confirms the direct effect of water stress on biological activities under climate change in summer. Moreover, as in winter, a lower aromaticity of soil organic matter fractions did not promote C-enzyme potential activities. Instead, we found that freePOM recalcitrance increased with higher C-enzyme potential activities (path relation 0.69 Fig. 4D) due to the fact that fresh plant material with less aromaticity chemistry was not yet decomposed under water stress condition in the summer under climate change (Gavazov et al., 2014). The accumulation of freePOM due to lower enzyme potential activities (Fig. 4B) adds further support for fresh plant material accumulation.

Interestingly, microbial community composition had no effect on SOM enzymatic composition, as reported by Schnecker et al. (2014). However, the representation of microbial community structure with PLFA data summarized using the two axis of the principal component analysis (Puissant et al., 2015) may not provide enough taxonomic resolution to correctly detect changes in microbial taxa which could influence soil enzyme potential activities under climate change conditions. Additionally, another factor may be that accelerated microbial processes rates and community shifts are likely to happen after a rain event within hotspots over short periods of time (Kuzyakov and al, 2015), particularly in summer when the system is under water stress. In this study, the one-time point sampling does not allow consideration of such events, possibly obscuring underlying interactions between microbial community structure and substrate chemistry.

5. Conclusion

Overall, our results clearly demonstrate two distinct effects of a climate change manipulation (reduced precipitation and temperature increase) in winter and summer seasons in subalpine grassland. Soil

moisture change induced by the climate change manipulation decreased C-enzyme activities by reducing substrate availability (WEOC) in winter and by decreasing microbial biomass under water stress condition in summer. Our results provide a comprehensive picture of potential seasonal cause and effect relationships governing C mineralization in subalpine grasslands exposed to a natural climate change scenario. This knowledge will allow better understanding of future changes in soil processes under climate change in subalpine ecosystems, and permit better predictions of the likely future impact on soil ecosystem services.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2018.06.023>.

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